

## Prevalence, intensity and influence of size and seasons on parasites of *Oreochromis niloticus* in Ekiti State dams, Southwest, Nigeria

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### Abstract

Fish are subject to various contaminants and environmental contradictions in the aquatic environment that convey stress on them, making them vulnerable to parasites. Therefore, this study was designed to investigate the prevalence and intensity of parasitic infections in *Oreochromis niloticus* that are of dietary and financial values in Ekiti State. 540 specimens of *O. niloticus* with different lengths and weights were indiscriminately procured from fishermen at Egbe, Ero and Ureje dams, Ekiti state between November, 2018 and October, 2019. Standard techniques for parasitology were employed in the study. Fish samples were examined, dissected and parasites were removed, identified and counted. Total parasites of 308 (16 genera), 275 (10 genera) and 468 (13 genera) were collected from Egbe, Ero and Ureje dams respectively. The total parasite prevalence was 53.3, 58.3 and 55.5% for Egbe, Ero and Ureje dams respectively. In this investigation, parasite prevalence associated significantly at  $P < 0.05$  with body length and weight in Egbe, Ero and Ureje dams. This indicates the procurement of parasite by age. The mean intensity and abundance of parasites in the three dams were higher significantly at  $P < 0.05$  in rainy season in comparison to dry season. Irrespective of seasons, the parasite abundance and the mean intensity were higher significantly at  $P < 0.05$  in Ureje dam in comparison to Egbe and Ero dams. High degree of pollution normally sustain parasite load as indicated in this study and could diminish fish performance and production. Hence, pollution control and regular examination of the water bodies should be advocated.

**Keywords:** *Oreochromis niloticus*, dams, parasites, prevalence, intensity.

### INTRODUCTION

Fishes are vital to man as they function as a good source of animal protein for both man and livestock. They also function as a source of earnings in Nigeria and other countries in sub-Saharan Africa where many people depend exclusively or partially on the fisheries division for their sustenance (FAO, 1996). Parasites generally affect the market value of fish and, therefore, are of community health concern. In order to obtain healthy and quality fish meat, the fish should be devoid of all types of infections. As precious as our dams and other aquatic bodies are as sources for fish production, only a few of them are in their natural healthy conditions and have become of great concern in terms of water quantity and quality (Bash *et al.*, 2001; Boyd, 2003). An aquatic ecosystem has several sources of pollution, ensuing from human activities such as industrial procedures, increased urbanization, and waste release (Aladaileh *et al.*, 2020). Many ailments found in fish are strictly connected to environmental degradation and stress. Fish, given the fact that they naturally thrive and get nourished within the water environment, display certain restrictions in evading the dangerous effects of pollutants (Ahmed *et al.*, 2020), are subject to diverse pollutants

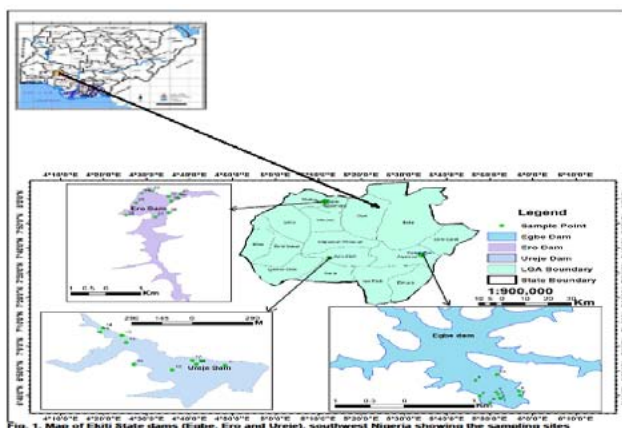
and environmental inconsistencies in the aquatic environment that impart stress on them, making them susceptible to pathogens. Due to a decline in immunological defenses and a lower susceptibility to infections, environmental pollutants might cause a rise in fish parasites. The most significant influences on parasite prevalence are water quality and season (Authman *et al.*, 2008). Pollutants have been shown to increase parasitism in aquatic species, particularly fish (Oros and Hanzelova, 2009). A decrease in the level of water oxygen, an upsurge in water organic matter, and degraded environmental conditions are all factors that prompt parasitic infestations in fish species. The category and quantity of parasites present have a significant influence on the impact of parasitic infection on fish species. Fish parasites are superb biological pointers of environmental quality, as their parasitic populations may grow or decline in response to alterations in water factors and/or the existence of pollutants. Hence, fish parasites are useful as biological indicators to illustrate the ecology of their infected hosts. The greater demand for fish as a better source of animal protein has made the need for obtaining healthy fish through the continuous study of fish fauna and fish parasites of paramount importance. Therefore, this study aimed at examining

the prevalence and intensity of parasitic infections in *Oreochromis niloticus* that are of nutritional and economic value to the Ekiti State populace especially the rural dwellers.

## Materials and methods

### Study area

Ekiti State is geographically located between longitude  $4^{\circ} 45'$  to  $5^{\circ} 45'$  East of the Greenwich Meridian and latitude  $7^{\circ} 15'$  to  $8^{\circ} 5'$  North of the equator. It is bordered on the north by Kwara State, on the east by Kogi State, and on the south and east by Osun State. Ekiti State dams lie within the southwestern basement complex of Nigeria. This study involves the three major dams in the state: Egbe Dam, situated at Longitude  $5^{\circ} 36.91$  E and Latitude  $7^{\circ} 37.11$  N; Ero Dam, situated at Ikun-Ekiti, Moba Local and Ureje Dam, located at Longitude  $5^{\circ} 141$  E and Latitude  $7^{\circ} 38.21$  N (Figure 1). Human activities such as defecation, washing, recreation (boating, swimming/bathing, and fishing), agricultural activities, and waste disposal are prevalent, especially where human settlements exist around the dams. The various agricultural and domestic activities occurring within the dams constantly involve the use of agrochemicals and insecticides to boost their production and combat insect pests respectively. Villages and families in the dam's vicinity dump domestic solid and liquid wastes straight into the dams without treatment, while farm runoff is carried directly into the dams. The utilization of fisheries in these dams has a direct impact on employment, income, and nutritional status of the local people who live around these dams and their catchments.



**Fig. 1.** Map of Ekiti State dams (Egbe, Ero and Ureje), southwest Nigeria.

### Sample collection

A cross-sectional study that involved fish sampling for parasitological examination and the water quality evaluation were carried out from November 2018

to October 2019 in three major dams in Ekiti State- Egbe, Ero and Ureje dams. Samples of fish were collected monthly for 12 months. Fish samples were collected in the morning between 8:00 and 10:00 a.m. A total of 540 fish samples were collected using gill nets with the assistance of fishermen operating in the dams. One hundred and eighty fish samples were collected from each dam. Fish were labeled, numbered and transported in ice packed container to laboratory and additional investigations were immediately conducted on them.

### Laboratory analysis of fish samples

#### (i) Length-weight measurement

Moisture was removed from the fish (*Oreochromis niloticus*) with blotting paper. The measurement of fish samples total length in cm and weight in grammes was carried out. The fish weights were measured to the nearest gramme with a digital top loading weighing scale, Ohaus CS 5000 model and the lengths (total and standard) were taken to the nearest 0.1 cm with meter rule. Sexes of the fish were obtained after dissection.

#### (ii) Parasite assessment

The fins, opercula, mouth and skin of fish samples were checked for external parasites using hand lens. The fish specimens were placed in a tray and examined grossly for occurrence of parasites. Areas between the fins, under the pelvic and pectoral fins were examined. Mucus scrapings from various parts of the body were placed on a petri dish, to which a small quantity of saline solution was added and was examined for parasites with a light microscope. The different organs, such as skin, gills, opercula, eyes, muscle, liver, kidney, spleen, swim bladder and the digestive tract were investigated for endoparasites by placing them separately in petri dishes containing saline solution. The intestinal tracts were cautiously opened and divided into parts which were later cut open, washed and cleaned in petri bowls containing 0.1% solution of NaCl and 0.1%  $\text{NaHCO}_3$  respectively. It was then stained with Giemsa stain and viewed at x 40 and x 100 magnification for the presence of endoparasites. In addition, encysted parasites were meticulously searched for in the muscles. The dissected gills were placed in saline solution in petri dishes. Droplets of the saline solution were applied on a microscope slide and viewed at x 40 and x 100 amplification for parasites in the gills. If found, parasites were removed and identified while the photographs of the microscopic ones were taken with camera connected to a microscope and viewed on the laptop. Examination of the fish for parasites and identification of parasites was according to

standard procedures (Paperna, 1996). The parasites were identified and counted. The abundance, mean intensity and prevalence of parasitic infestation were computed using the method of Margolis *et al.* (1982) and Bush *et al.* (2001).

$$\text{Prevalence of infection} = \frac{\text{Infested host}}{\text{Total number of host examined}} \times 100$$

$$\text{Incidence of infection} = \frac{\text{No of parasites collected in a sample}}{\text{No of infested host}}$$

$$\text{Prevalence of individual parasite} = \frac{\text{No of parasites collected in a sample}}{\text{No of infested host}} \times 100$$

### Statistical analysis

The association that occurs between the parasite burden and length, weight and seasons were related using correlation analysis and t-test. Significant values were taken at  $P \leq 0.05$ . Analysis of Variance was used to assess the parasite load among the dams.

### Results

#### Parasite Spectrum of *Oreochromis niloticus* in the dams

The species of parasites isolated from *O. niloticus* are shown in Table 1. Total parasites of 308 (16 genera), 275 (10 genera) and 468 (13 genera) were collected from Egbe, Ero and Ureje dams respectively (Table 2). The total parasite prevalence was 53.3, 58.3 and 55.5% for Egbe, Ero and Ureje dams respectively. *Dactylogyrus* was the most prevalent parasite in Egbe dam while *Amrithalingamia macracantha*, *Piscinoodinium pillulare* and *Eurostronglodes tubifex* were the most prevalent parasites in Ero dam. In Ureje dam, *Piscinoodinium pillulare* showed the highest prevalence. *Trichodina mutabillis*, *Piscinoodinium pillulare*, *Camallanus polypteri* and *Capillaria pterophylli* occurred in the fish from all the dams, *Chidonella piscicola* were obtained in fish from Ureje and Egbe dams while *Amrithalingamia macracantha*, *Bolbophorus levanticus* were found in fish from Egbe and Ero dams. *Ichthyophthirius multifiliis*, *Myxobolus tilapiae*, *Loma camerounensis* and *Contracaecum spiculigerum* occurred in fish from Ureje and Ero dams.

In Egbe dam, mean abundance was 1.71 parasites per fish specimen examined with mean intensity up to 3.21 parasites per specimen infected; in Ero dam, the

mean abundance is 1.53 with mean intensity of 2.62 and in Ureje dam, the mean abundance is 2.60 with the mean intensity of 4.73 (Table 2). Table 3 shows the prevalence of parasites in *O. niloticus* in relation to fish length. In Egbe dam during the dry season, the highest prevalence of 7 (70.00%) was obtained in *O. niloticus* within the total length range of 20.0 - 21.9 cm; the highest prevalence of 1(100%) was obtained in fish in Ero dam within the total length range of 20.0 - 21.9 cm while in Ureje dam, the highest prevalence of 1 (100%) was obtained in fish total length range of 20.0 - 21.9 cm. During the rainy season, the highest prevalence of 7 (77.78%) was obtained in *O. niloticus* in Egbe dam within the total length range of 20.0 - 21.9 cm; the highest prevalence of 20 (74.07%) was obtained in fish in Ero dam within the total length range of 20.0 - 21.9 cm while in Ureje dam, the highest prevalence of 13 (72.22%) was obtained in fish total length range of 20.0 - 21.9 cm.

Prevalence of *O. niloticus* in relation to the body weight is shown in Table 4. The highest prevalence of 1 (100%) in Egbe dam during the dry season was in fish with body weight of 120-139g, in Ero dam, the highest prevalence of 15 (71.43%) was found in fish with body weight range of 140-159g while in Ureje dam, the highest prevalence of 11 (61.11%) was obtained in fish with body weight range of 160-179g. During the rainy season, the highest prevalence of 27 (75.00%) in Egbe dam was found in fish with weight range of 140-159g; the highest prevalence of 25 (69.44%) was found in fish with body weight range of 120-139g in Ero dam while in Ureje dam, the highest prevalence of 33(70.21%) was found in fish with body weight range of 160-179g. In this investigation, it was witnessed that parasite prevalence associated significantly at  $P < 0.05$  with body length and weight in Egbe, Ero and Ureje dams (Tables 5).

Table 6 shows the prevalence of parasites, the mean intensity and abundance in the dams in dry and rainy periods. The mean intensity and abundance of parasites in the three dams were higher significantly at  $P < 0.05$  in rainy season in comparison to dry season. Irrespective of seasons, the parasite abundance and the mean intensity were higher significantly at  $P < 0.05$  in Ureje dam in comparison to Egbe and Ero dams.

**Table 1:** Parasitic Infections in *O. niloticus* Sampled from Egbe, Ero and Ureje Dams

Parasite	Egbe dam				Ero dam				Ureje dam			
	TP	P%	MI	A	TP	P%	MI	A	TP	P%	MI	A
<b>Protozoa</b>												
<i>Chilodonella piscicola</i>	48	5.00	5.33	0.27	-	-	-	-	35	1.70	11.67	0.19
<i>Ichthyophthirius multifiliis</i>					32	5.00	3.56	0.18	30	3.30	5.00	0.17
<i>Trichodina mutabilis</i>	85	8.30	5.67	0.47	25	5.00	2.78	0.14	46	5.00	5.11	0.26
<i>Cryptobia iubilans</i>	9	1.70	3.00	0.05	-	-	-	-	-	-	-	-
<i>Ichtyobodo costia</i>	-	-	-	-	9	1.70	1.50	0.05	-	-	-	-
<i>Tetrahymena bergeri</i>	-	-	-	-	-	-	-	-	91	3.30	15.17	0.51
<i>Ambiphrya ameiuri</i>	-	-	-	-	-	-	-	-	41	3.30	6.83	0.23
<i>Piscinoodinium pillulare</i>	62	6.70	5.17	0.34	55	6.70	4.58	0.31	132	16.70	4.40	0.73
<i>Myxobolus tilapiae</i>	-	-	-	-	23	3.30	3.83	0.13	9	1.70	3.00	0.05
<i>Loma camerounensis</i>	-	-	-	-	28	3.30	4.67	0.16	8	1.70	2.67	0.04
<i>Goussia cichlidarum</i>	-	-	-	-	-	-	-	-	8	1.70	2.67	0.04
<b>Monogenea</b>												
<i>Gyrodactylus cichlidarum</i>	22	8.30	1.47	0.12	-	-	-	-	-	-	-	-
<i>Dactylogyrus baueri</i>	30	13.30	1.25	0.17	-	-	-	-	-	-	-	-
<b>Digenea</b>												
<i>Clinostomum tilapiae</i>	-	-	-	-	11	1.70	3.67	0.06	-	-	-	-
<i>Aloocreadium sp</i>	-	-	-	-	8	3.30	1.33	0.04	15	6.70	1.25	0.08
<i>Bolbophorus levanticus</i>	8	1.70	2.67	0.04	7	3.30	1.17	0.04	-	-	-	-
<i>Leithochirium floridense</i>	-	-	-	-	5	1.70	1.67	0.03	-	-	-	-
<b>Cestodes</b>												
<i>Amrithalingamia macracantha</i>	10	3.30	1.67	0.06	16	6.70	1.33	0.09	-	-	-	-
<i>Diphyllobothrium latum</i>	-	-	-	-	5	1.70	1.67	0.03	-	-	-	-
<b>Nematodes</b>												
<i>Camallanus polypteri</i>	18	1.70	6.00	0.10	10	1.70	3.33	0.06	12	1.70	4.00	0.07
<i>Capillaria pterophylli</i>	16	3.30	2.67	0.09	14	3.30	2.33	0.08	25	6.70	2.08	0.14
<i>Eustrongyloides tubifex</i>	-	-	-	-	14	6.70	1.17	0.08	-	-	-	-
<i>Contracaecum spiculigerum</i>	-	-	-	-	6	1.70	2.00	0.03	16	1.70	5.33	0.09
<i>Ascaris lumbricoides</i>	-	-	-	-	7	1.70	2.33	0.04	-	-	-	-

TP = Total no. of individual parasites, P% = Percentage prevalence, MI = Mean intensity, A = Abundance

**Table 2:** Prevalence, Mean Intensity and Abundance of Parasitic Infestation of *O. niloticus* in Egbe, Ero and Ureje Dams

Dam	Number of Hosts		Total no of parasites	Prevalence (%)	Mean Intensity	Abundance
	Examined	Infected				
Egbe	180	96	308	53.33	3.21 ± 0.16 <sup>a</sup>	1.71 ± 0.15 <sup>a</sup>
Ero	180	105	275	58.33	2.62 ± 0.12 <sup>b</sup>	1.53 ± 0.12 <sup>b</sup>
Ureje	180	99	468	55.00	4.73 ± 0.22 <sup>c</sup>	2.60 ± 0.21 <sup>c</sup>
Total	540	300	1051	55.56	3.50 ± 0.11	1.95 ± 0.10

Mean intensity and abundance with different alphabet superscriptions are significantly different at P < 0.05.

**Table 3:** Prevalence and Seasonal Dynamic of Detected Parasites in Egbe, Ero and Ureje Dams Based on Total Length of *O. niloticus*

Total length (cm)	Dry season									Rainy season								
	Egbe dam			Ero dam			Ureje dam			Egbe dam			Ero dam			Ureje dam		
	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)
12.0 -13.9	20	6	30.00	10	5	50.00	4	-	-	3	1	33.33	3	1	33.33	-	-	-
14.0 - 15.9	25	9	36.00	32	11	34.38	31	11	35.48	37	13	35.14	9	5	55.56	9	4	44.44
16.0 - 17.9	20	13	65.00	38	25	65.79	20	9	45.00	3	2	66.67	25	12	48.00	54	35	55.56
18.0 - 19.9	15	10	66.67	9	7	77.78	34	25	73.53	38	28	73.68	26	18	69.23	9	5	64.81
20.0 - 21.9	10	7	70.00	1	1	100.00	1	1	100.00	9	7	77.78	27	20	74.07	18	13	72.22

NEF: Number of fish examined fish; PF: parasitized fish; P: Percentage prevalence

**Table 4:** Prevalence and Seasonal Dynamic of Detected Parasites in Egbe, Ero and Ureje Dams based on the Weight of *O. niloticus*

Weight (g)	Dry season									Rainy season								
	Egbe dam			Ero dam			Ureje dam			Egbe dam			Ero dam			Ureje dam		
	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)	NEF	PF	P (%)
40 – 59	3	1	33.33	3	-	-	-	-	-	9	1	11.11	3	1	33.33	-	-	-
60 – 79	39	16	41.03	9	3	33.33	6	1	16.67	9	3	33.33	3	1	33.33	-	-	-
80 – 99	14	6	42.86	15	8	53.33	12	5	41.67	12	3	25.00	39	24	61.54	3	1	33.33
100 – 119	33	21	63.64	15	6	40.00	12	6	50.00	9	6	66.67	9	5	55.56	9	3	33.33
120 – 139	1	1	100	6	4	66.67	15	6	40.00	15	11	73.33	36	25	69.44	3	2	66.67
140 – 159	-	-	-	21	15	71.43	27	13	48.15	36	27	75.00	-	-	-	28	18	64.29
160 – 179	-	-	-	21	13	61.90	18	11	61.11	-	-	-	-	-	-	47	33	70.21

NEF: Number of fish examined fish; PF: parasitized fish; P: Percentage prevalence

**Table 5:** Correlations between Parasite Prevalence and Fish Total Length and Body Weight in Egbe, Ero and Ureje Dams during Dry and Rainy Seasons

	Dry season	Prevalence	Significance	Rainy season	Prevalence	Significance
Length	Egbe dam	0.923	0.025	Egbe dam	0.939	0.018
	Ero dam	0.898	0.038	Ero dam	0.916	0.029
	Ureje dam	0.981	0.019	Ureje dam	0.996	0.004
Weight	Egbe dam	0.900	0.037	Egbe dam	0.930	0.007
	Ero dam	0.856	0.014	Ero dam	0.902	0.036
	Ureje dam	0.832	0.040	Ureje dam	0.891	0.043

**Table 6:** Prevalence, Mean Abundance and Intensity of Parasite According to Season in Egbe, Ero and Ureje Dams

Season	Dam	NFE	NIF	P (%)	TP	Abundance	MI
Dry season	Egbe	90	45	50	109	1.21 ± 0.15 <sup>a</sup>	2.42 ± 0.14 <sup>a</sup>
	Ero	90	49	51.11	99	1.10 ± 0.12 <sup>a</sup>	2.06 ± 0.11 <sup>a</sup>
	Ureje	90	42	46.67	140	1.56 ± 0.22 <sup>b</sup>	3.33 ± 0.29 <sup>b</sup>
Rainy season	Egbe	90	51	56.67	199	2.21 ± 0.25 <sup>a</sup>	3.90 ± 0.24 <sup>a</sup>
	Ero	90	57	65.56	176	1.96 ± 0.19 <sup>b</sup>	3.09 ± 0.18 <sup>b</sup>
	Ureje	90	57	63.33	328	3.64 ± 0.33 <sup>c</sup>	5.75 ± 0.25 <sup>c</sup>

NFE: Number of fish examined, NIF: Number of infected fish, P%: Prevalence, TP: Total number of parasites, MI: Mean intensity. Mean intensity and abundance with different alphabet superscriptions are different significantly at P < 0.05.

## Discussion

Information on parasitic contaminations in fish is linked to fish health and understanding of ecological issues (Sures *et al.*, 2017). The five parasitic groups (protozoa, monogenea, digenea, cestoda, and nematoda) observed in this study are comparable to the observations of Ashade *et al.* (2013) in their study on parasites of *O. niloticus* from three selected water bodies in Lagos State, Southwest Nigeria. The highest parasite abundance and mean parasite intensity in fish from Ureje dam than in fish from Egbe and Ero dams may be related to the poorest state of the dam, as shown by the water quality index (Olagbemide and Owolabi, 2019). Adewole *et al.* (2018) documented a parallel report during their research on parasites as a bio-indicator to assess the health and ecological position of fish species in freshwater in Ekiti State, and they associated the parasite abundance with the polluted state of the dams.

The total prevalence in each of the dams as revealed in this study was higher than 29.0% and 47.8% found by Omoniyi and Ojelade (2017) and Amare *et al.* (2014) respectively for *O. niloticus*; but lower than 100.00% reported by Mitiku *et al.* (2018) in the same species from Lake Koftu in central Ethiopia and 77.60% observed by Areda *et al.* (2019) from selected fish farms in south west zone, Oromia region, Ethiopia. However, the prevalence was within the range of 57.3% reported by Abiyu *et al.* (2020) in the species caught from south west part of Lake Tana, Central Gondar, Ethiopia. Prevalence, therefore, appeared to vary greatly from one locality to the other and this may be due to factors such as endemicity, availability of intermediate hosts and susceptibility of host to infection (Chandler and Read, 1981). The relatively higher prevalence of parasites, mean abundance and the mean intensity in the rainy period when compared with dry period in all the dams was likely due to the poorer water quality, higher metals and OCPs concentrations in the dams in the rainy period in comparison to dry period. Similar reports were given Sinarř *et al.* (2013).

Thus, this supports the assertion that increase parasitism in aquatic biota especially fish may be promoted by pollutants (Oros and Hanzelova, 2009). The different in parasites number and genera observed in the fish from the three dams might be due to the differences in physico-chemical parameters expressed in water quality indices in the dams (Olagbemide and Owolabi, 2019).

The observed significant correlation of parasite prevalence with the length and weight of fish from the dams in this study were also documented by many other investigators. This is an indication that procurement of parasite is by age. Bichi and Ibrahim (2009); Ayanda (2009), Allumma and Idowu (2011); Akoll *et al.* (2012), Saha *et al.* (2015) and Atalabi *et al.* (2018) reported that bigger fishes were severely infected by parasites when compared with smaller ones and they attributed this to the extended period of time the grown-up fish were vulnerable to the pollutants in the surroundings which intensifies their likelihoods of procuring parasite infection with time and the ability of larger fish to offer larger outward area used for infection in comparison to smaller fish while Esch and Fernandez (1993); Paperna, (1996) and Marcogliese, (2002) related it to their ontogenetic habitat and feeding shifts. However, others workers like Akinsanya *et al.* (2007), Biu and Nkechi (2013); and Biu *et al.* (2014) documented that the younger fish were more diseased in comparison to the older ones and they attributed it to the reality that younger fish have less resistance contrary to parasites while older fish have completely developed resistance contrary to parasitic infestation while other worker such as Amaechi (2015) found no relationship between parasite burden and length and weight of fish.

## Conclusion

Fish parasitism creates a key menace to fish production and high degree of pollution normally sustenance parasite load as indicated in this study and could diminish fish performance and production. Hence, pollution control and regular examination of the water bodies should be advocated.

## Declarations

**Ethical approval and consent to participate:** Ethical approval was obtained from Ethics Committee of the University of Ilorin and permission was obtained from Ekiti State Water Corporation for this study.

**Consent for publication:** Not applicable

**Competing interests:** Authors declared no conflict of interest.

**Availability of Data and Materials:** All data generated or analysed during this study are included in this published article

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**Authors' contributions:** OPT conceived and designed the experiment; data collection, analysis and interpretation; drafting of the article, designed figures and wrote the manuscript in consultation with OOD. OOD supervised the project; critical review of the article and final approval of the version to be published.

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